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Synthesis, Spectra and Antibacterial Studies of Ni(II), Fe(II) and Co(II) Complexes of 5-(4-Chlorophenyl)-6-Ethyl-2,4-Pyrimidinediamine

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ABSTRACT:
5-(4-chlorophenyl)-6-ethyl-2,4-pyrimidinediamine (pyrimethamine) is a preventive and curative drug for the treatment of parasitic infections . Ni(II), Fe(II) and Co(II) complexes of pyrimethamine were synthesized. The melting point, yield, and colour of the metal complexes were determined. The complexes were characterized based on Uv-visible and Infrared spectrophotometric analysis. The complexes and the ligand were screened against *Nesseria gonorrhea* and *Proteus mirabilis* at 250, 125 and 65.25 mg/ml concentration. The increase in the melting point and change in the colour of the complexes suggests complexation. The absorption maxima in the electronic spectra of the complexes were assigned intra-ligand charge transfer (ILCT) and ligand to metal charge transfer. Infrared spectra of the Ni(II) and Fe(II) pyrimethamine complexes suggests the involvement of two NH_2 and two C=N functional groups in coordination to the metal. IR spectrum of Co(II) pyrimethamine complex the suggested the involvement of two NH_2 fuctionality in coordination to cobalt. The zone of inhibition of pyrimethamine against *Nesseria gonorrhea and Proteus mirabilisat at* 250.00, 125.00 and 65.25 mg/l showed that it was significantly lower ($P<0.05$) than the complexes. The zone of inhibition of Fe(II) pyrimethamine complex against *Nesseria gonorrhea and Proteus mirabilisat at* 250.00, 125.00 and 65.25 mg/l showed that it was significantly higher ($P<0.05$) than Ni(II) and Co(II) pyrimethamine complexes. This suggests that the complexes were more potent than the parent ligand against the bacteria strains used.
Key words: Pyrimethamine**,** antibacteria, complexes, spectroscopic, ligands

INTRODUCTION

Pyrimethamine is a preventive and curative drug for the treatment of parasitic infections[1,2]. Pyrimethamine can be administered in conjuction with sulphadoxine in the treatment of malaria and in the secondary prevention for people with HIV/AIDS [2]. The resistant of pyrimethamine in the treatment of malaria may be caused by mutation in the malaria gene for dihydrofolate reductase [3]. It is an important medication for primary healthcare and it is listed as World Health Organisation's list of essential medicine [4]. It is a dihydrofolate reductase (DHFR) inhibitor since it inhibits folic acid metabolism. The structure of pyrimethamine is shown in Figure 1.

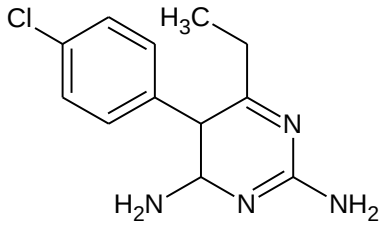

Figure 1: Structure of Pyrimethamine

Metal complexes play important role in medical science because they activate or biotransform their parent ligand/drug [5]. Antimicrobial activities of metal complexes have been reported by Shelke and Co workers [6]. They reported that the complexes exhibited enhanced antimicrobial activity than the parent ligand [6]. The research on transition metal complexes is unending because they possess pharmacological properties [7-9]. Structural modification of bio-ligands with metal ions are of interest because the metal ions increases the activity of the bio-ligands. Recent Research has shown significant progress in the use of chelated complexes to treat diseases [10]. The rapid spread of drug-resistant antibacterial worldwide has stimulated the search for new drugs to treat millions of people infected with the bacteria's. There is an urgent need for antibacterial with novel structures, modes of action or both to deal with the development of resistance to the drugs in current use. Previous research has shown that attaching organic drugs to metal-containing fragments could enhance their activity against diseases.

In our effort towards the development of metal based drugs, we decided to synthesize metal complexes of pyrimethamine, characterize these complexes and determine the inhibition of the bacteria activities on the complexed metal in comparison with the ligand. This synthetic strategy involves modification of the activity of pyrimethamine through the incorporation of a transition metal into the molecular structure. This modification in the chemical structure of pyrimethamine changes the pharmacological action of the drug. It is envisaged that this would lead to novel pharmacological models for antibacterial agents.

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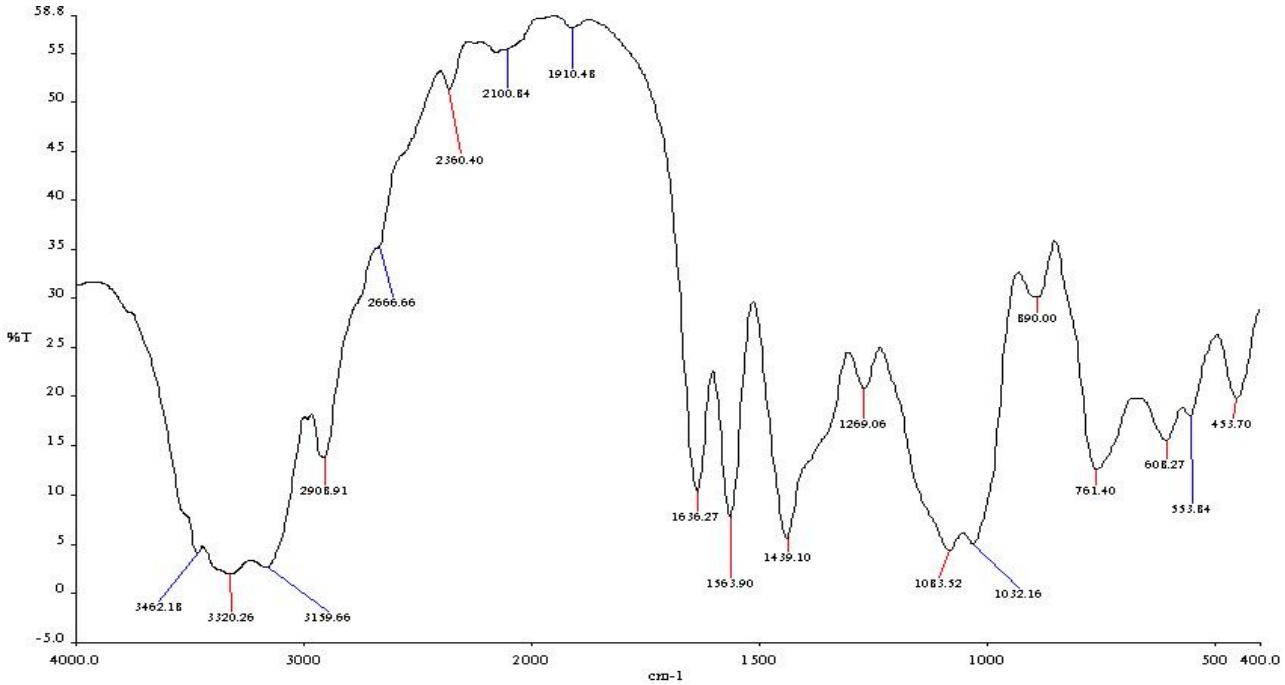

Figure 2: Infrared spectrum of pyrimethamine

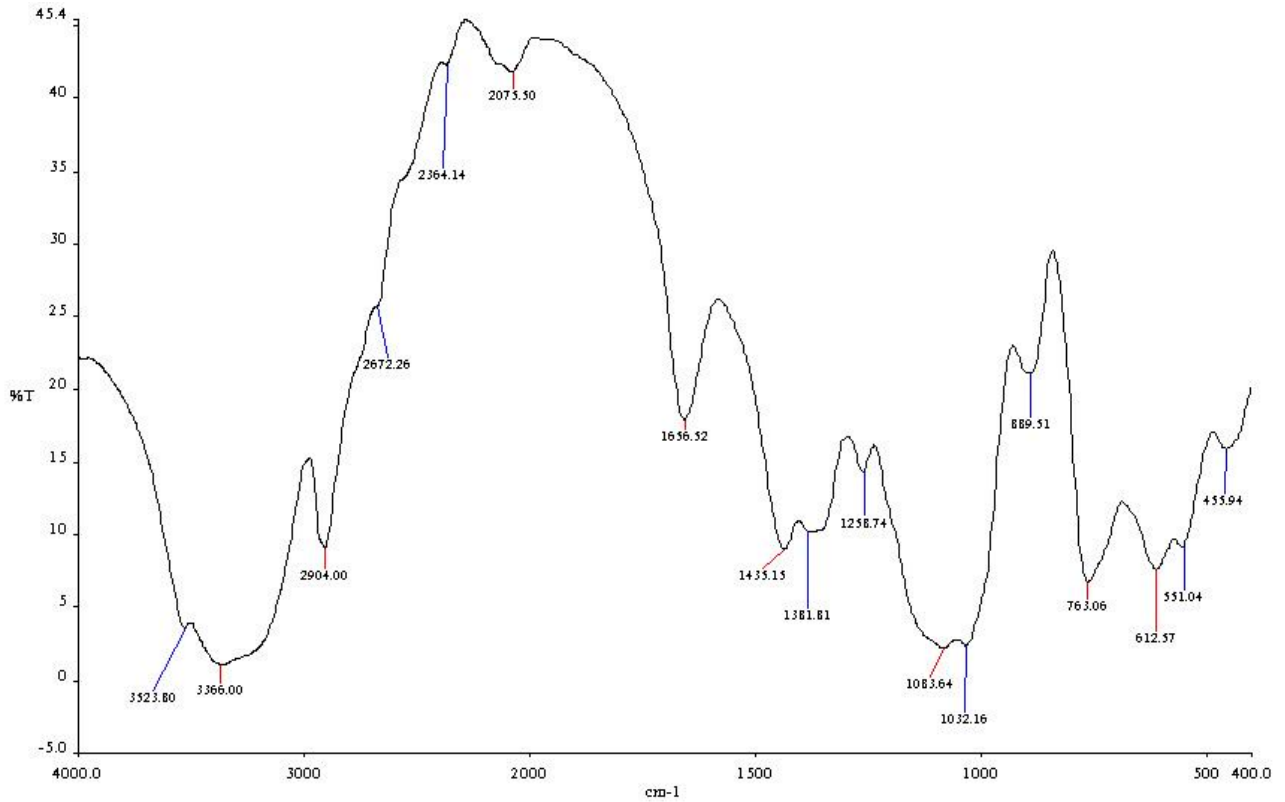

Figure 3: Infrared spectrum of pyrimethamine-Ni complex

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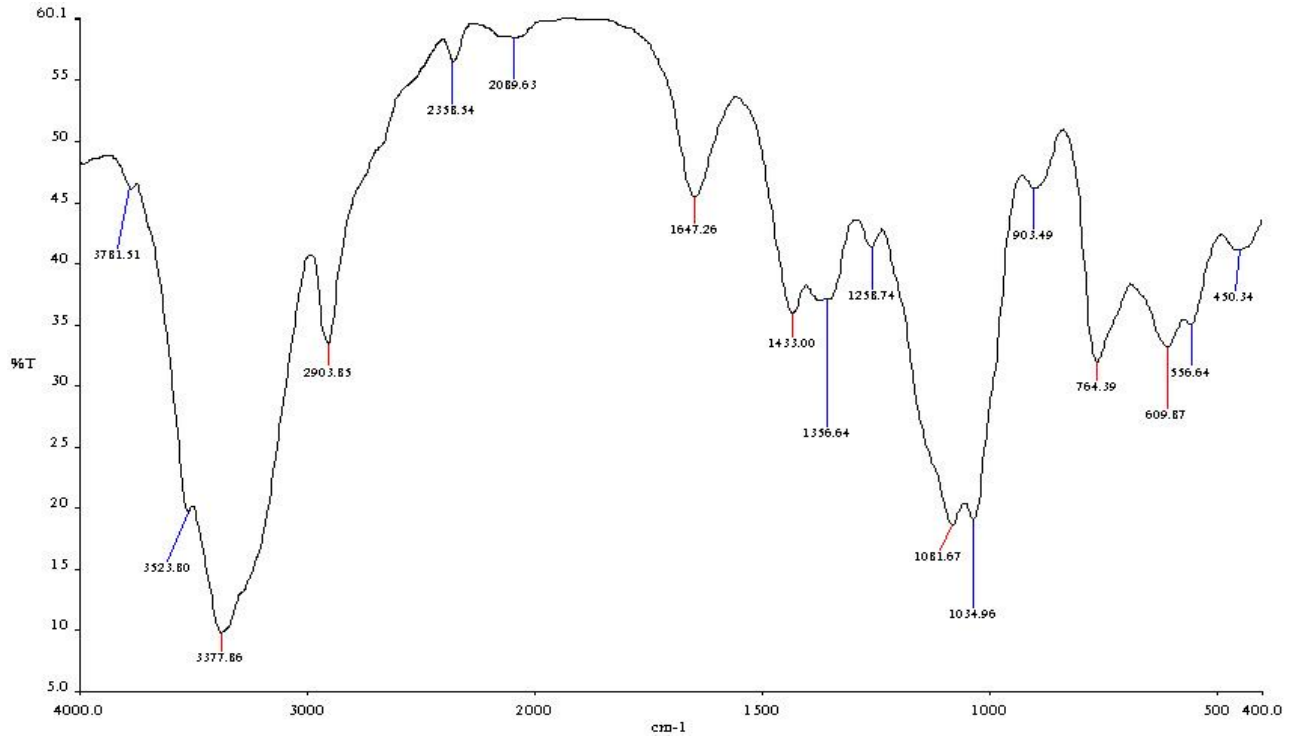

Figure 4: Infrared spectrum of pyrimethamine-Fe complex

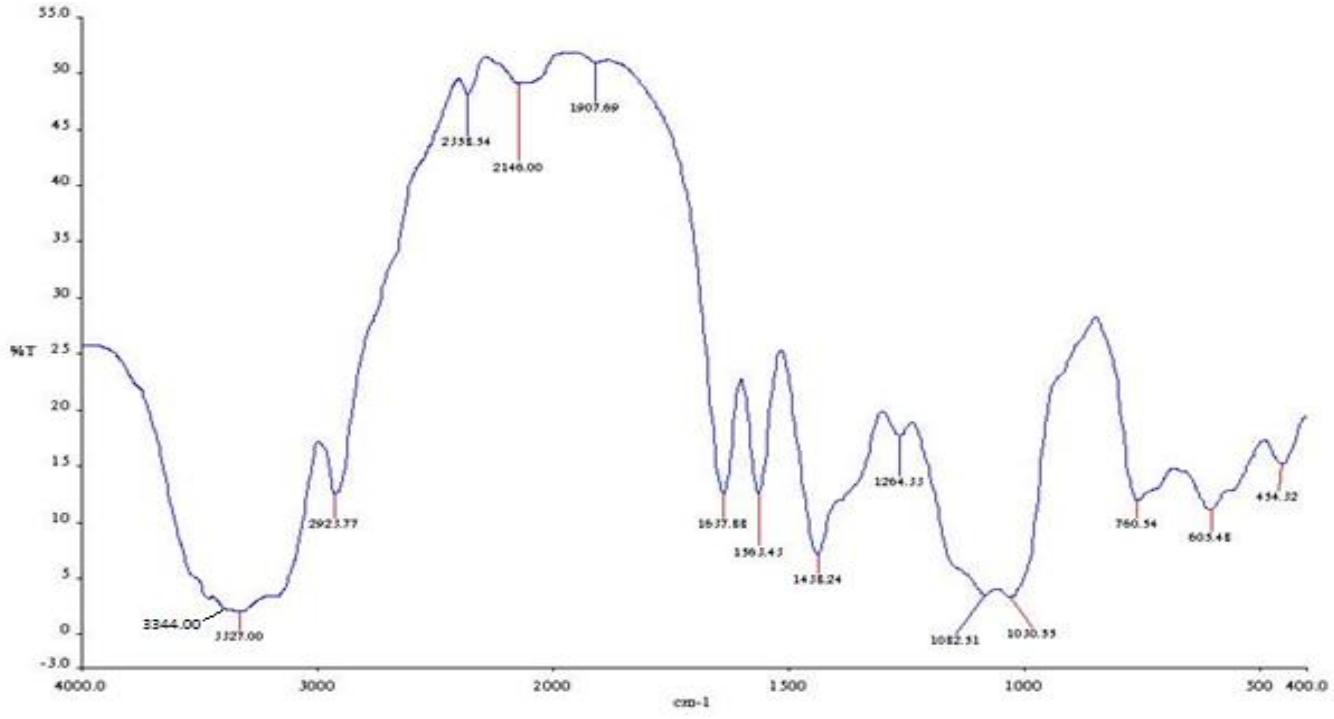

Figure 5: Infrared spectrum of pyrimethamine-Co complex

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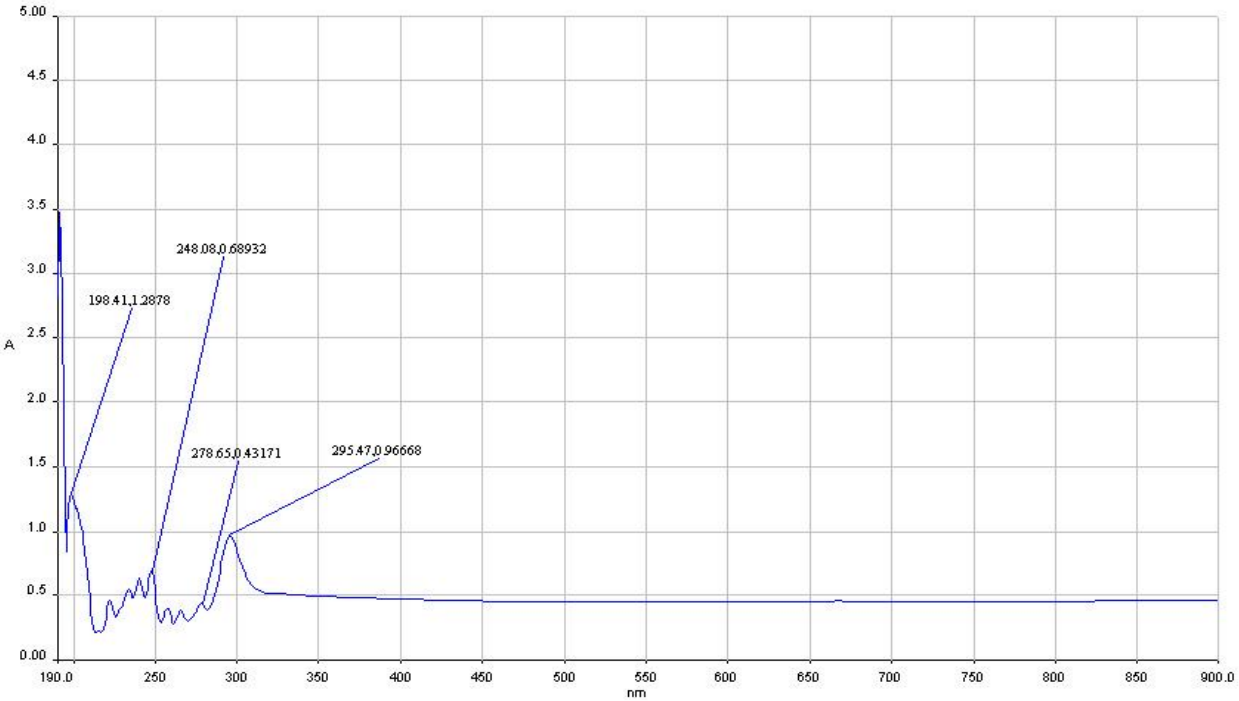

Figure 6: Electronic spectrum of pyrimethamine

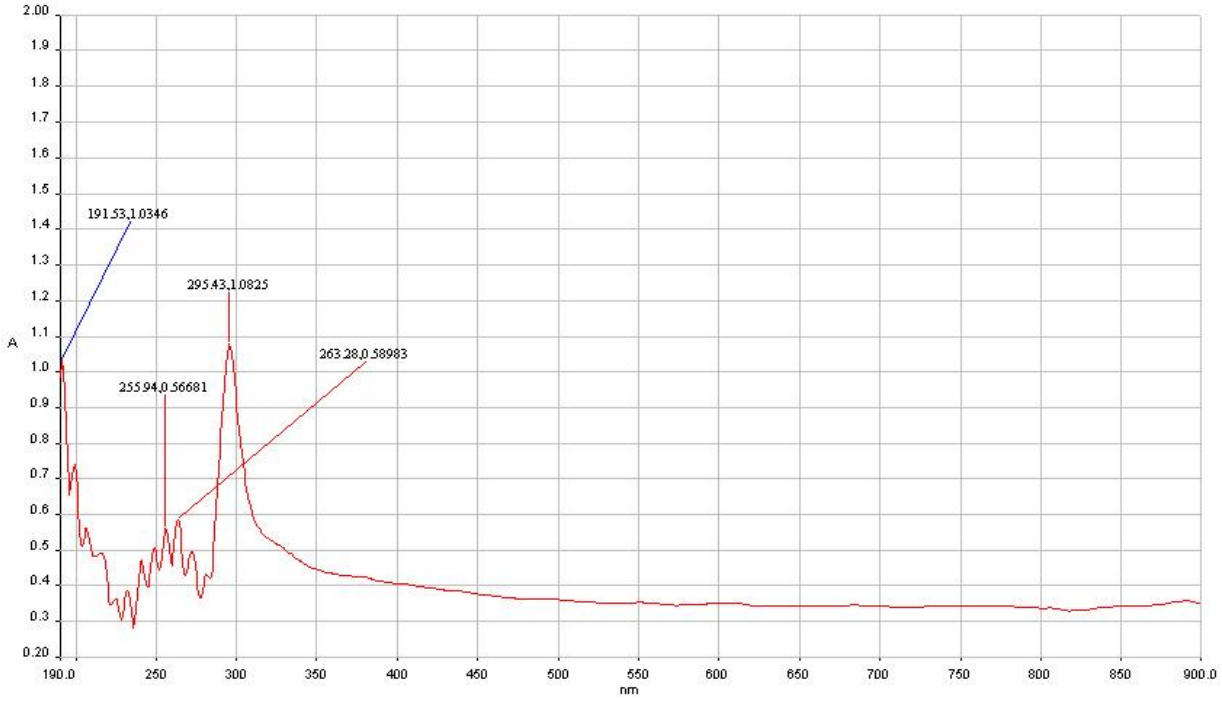

Figure 7: Electronic spectrum of pyrimethamine-Ni complex

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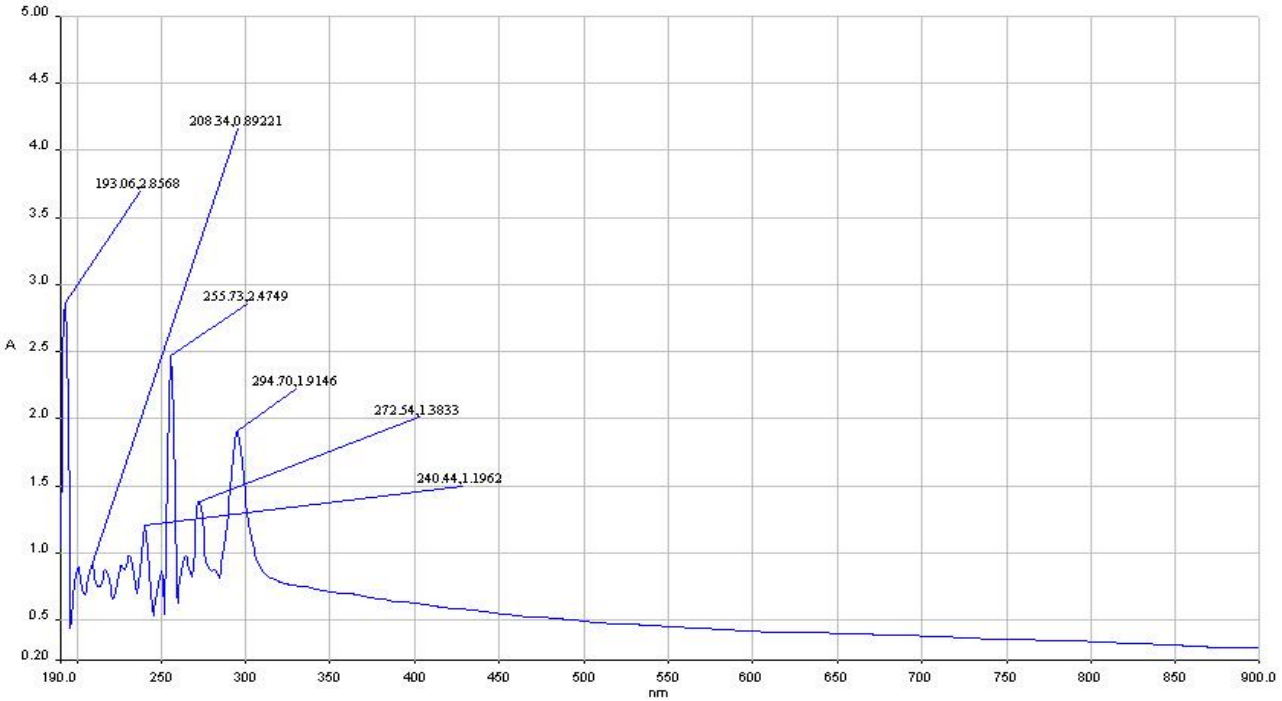

Figure 8: Electronic spectrum of pyrimethamine-Fe complex

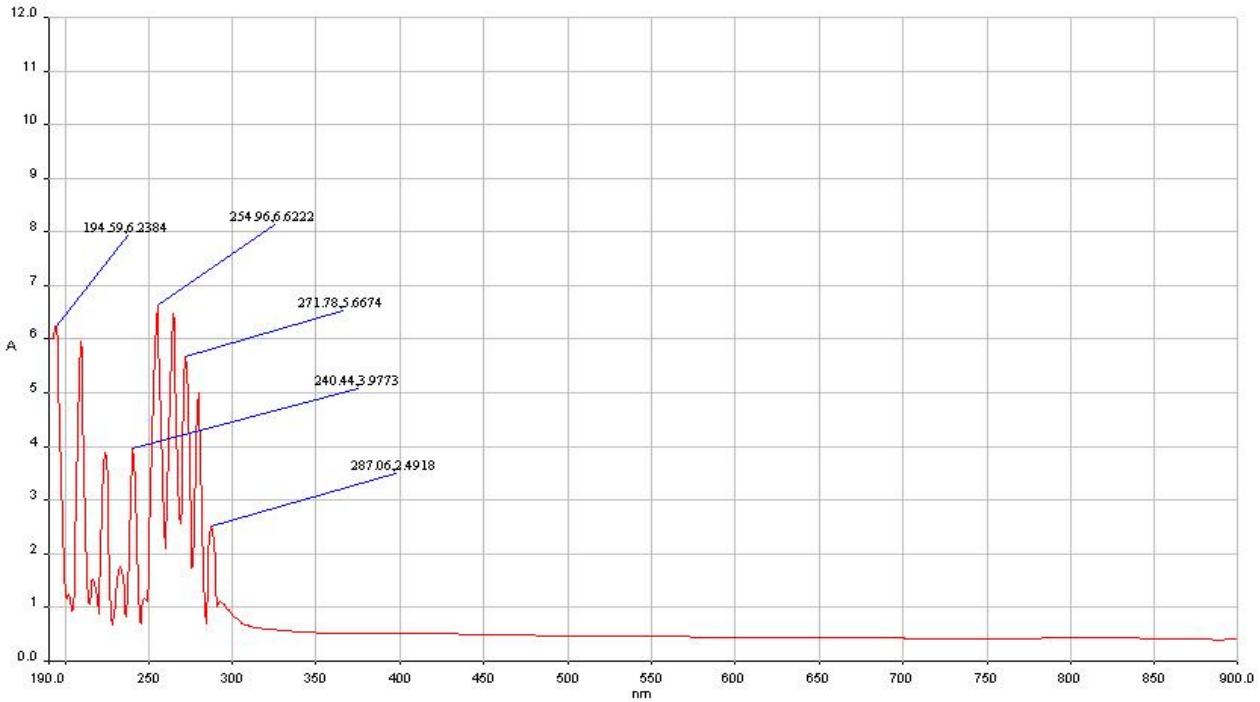

Figure 9: Electronic spectrum of pyrimethamine-Co complex

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Table 1: Some physical properties of the pyrimethamine and its complexes

Complexes	Melting point (^{0}C)	Yield (%)	Colour
$C_{12}H_{15}ClN_4$	207-212^{0}C	-	White
$[NiC_{12}H_{15}ClN_4]$	223^{0}C-230^{0}C	36%	Pale green
$[FeC_{12}H_{15}ClN_4]$	218^{0}C-223^{0}C	73%	Pale green
$[CoC_{12}H_{15}ClN_4]$	220^{0}C-229^{0}C	97%	Pale yellow

Table 2: Zone of inhibition of pyrimethamine and its complexes at 250.00 mg/l concentration

Bacterials	[FeL]	[NiL]	[CoL]	L
Nesseria gonorrhea	35.6±0.6^a	22.5± 0.5^b	15.1±0.96^c	9.3±0.6^d
Proteus mirabilisat	27.5±0.9^a	18.7±1.3^b	16.5± 1.4^c	8.3±0.3^d

L = $C_{12}H_{15}ClN_4$, Values are means ± standard deviation of four determination. Means with different superscript in the same shows that are significantly different (P<0.05) while means with the same superscript within the row do not differs significantly (P>0.05).

Table 3: Zone of inhibition of pyrimethamine and its complexes at 125.00 mg/l concentration

Bacterials	[FeL]	[NiL]	[CoL]	L
Nesseria gonorrhea	17.3±0.6^a	10.7±0.4^b	7.0±0.9^c	5. 0±0.2^d
Proteus mirabilisat	13.0±0.9^a	9.3±0.5^b	7.6±0.5^c	3.8 ±0.3^d

L = $C_{12}H_{15}ClN_4$, Values are means ± standard deviation of four determinations. Means with different superscript in the same shows that are significantly different (P<0.05) while means with the same superscript within the row do not differs significantly (P>0.05).

Table 4: Zone of inhibition of pyrimethamine and its complexes at 65.25 mg/l concentration

Bacterials	[FeL]	[NiL]	[CoL]	L
Nesseria gonorrhea	8.7±0.4^a	4.7±0.5^b	1.9±0.3^c	1.6±0.3^d
Proteus mirabilisat	6.9±0.9^a	4.0 ±0.2^b	3.0±0.2^c	0.5±0.6^d

L = $C_{12}H_{15}ClN_4$, Values are means ± standard deviation of four determinations. Means with different superscript in the same shows that are significantly different (P<0.05) while means with the same superscript within the row do not differs significantly (P>0.05)

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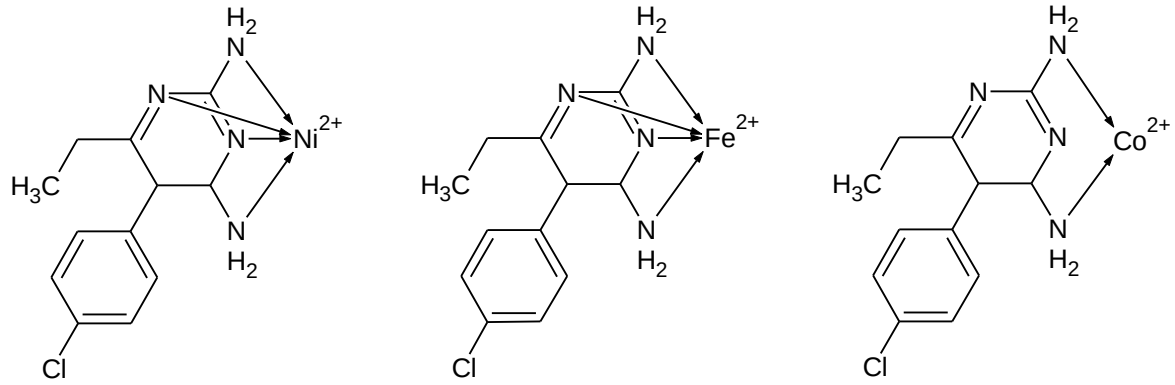

Figure 10: Proposed structures of pyrimethamine metal complexes

MATERIALS AND METHODS

All chemicals used in this study were of analytical grade and were used without further purification. 5-(4-chlorophenyl)-6-ethyl-2,4-pyrimidinediamine, $NiSO_4.6H_2O$, , $FeSO_4.7H_2O$, $Co(NO_3)_2$, methanol, chloroform, ethanol and distilled water were obtained from Sigma-Aldrich Chemical Company. The electronic spectra of the complexes in solution were scanned in the range 190-900nm on a Perkin Elmer 20λ Uv-visible spectrophotometer. The samples were dissolved in 5ml of chloroform then 1ml of each of the solution was re-dissolved in another 5ml chloroform. The solutions were the placed in Quartz cuvette of 1cm path length. The infrared spectra were collected on a 1000 FTIR Perkin Elmer spectrum Bx spectrophotometer equipped with caesium iodide window (4000-400cm^{-1}) in KBr pellets. The melting point of both the ligand and complexes were determined using capillary tube method.

Synthesis of pyrimethamine metal complexes

The solution of pyrimethamine was prepared by dissolving 10 mmol (2.487g) of pyrimethamine in 15ml of methanol. Solution of respective metal salt obtained by dissolving 5mmol of the metal salt, $NiSO_4.6H_2O$ (1.314 g); $FeSO_4.7H_2O$ (0.759 g); $Co(NO_3)_2$ (0.915 g) in 15ml of methanol. Pyrimethamine solution and the respective metal were stirred at 40^0C for 1 hour. After 1 hour the solutions were allowed to cool for 24 hours for the reaction to go to completion. The product formed were filtered off and washed with distilled water and finally dried at room temperature.

Antibacterial Susceptibility Testing

Antibacteria, activity of the extract was evaluated using the following organisms; *Nesseria gonorrhea* and *P.mirabilis*. The ability of the ligand and complexes to inhibit the growth of the bacteria was determined using agar-diffusion technique. Sterile glass pipettes of 8 mm diameter were used to make holes on prepared agar medium. Aliquots of 0.2 ml of the metal complex was introduced in the holes made on pre-seeded appropriate gelled media containing each isolate of organism, at different concentrations. Three plates were prepared, each containing four holes of 8 mm diameter for the different concentrations of the metal complex, for a specific isolate. In total, 14 petri-dishes were used and plates were incubated at 37^0C for 24 hours in the incubator.

RESULTS AND DISCUSSION

The melting point of pyrimethamine and its complexes are shown in Table 1. The infrared spectra of pyrimethamine and the complexes are shown in Figures 2-5. The electronic spectra of pyrimethamine and its complexes are presented in Figures 6-9. The antibacterial zone of inhibition of pyrimethamine and its complexes are shown in Tables 2-4.

The increase in the melting point of the complexes suggests complexation. The changes in the colour of the complexes further support that coordination occurred since transition metal are coloured.

In the infrared spectrum of pyrimethamine, the N-H vibrational streching frequency appearred at 3320.26 cm^{-1} but in the spectra of the metal complexes $[NiC_{12}H_{15}ClN_4]$, $[FeC_{12}H_{15}ClN_4]$ and $[CoC_{12}H_{15}ClN_4]$ it appeared at 3366.00, 3377.66 and 3340.00 cm^{-1} respectively. This suggests that N-H functional group was involved in coordination to the metal ions. C=N vibration stretching frequency appeared at 1636.27cm^{-1} in pyrimethamine but in the $[NiC_{12}H_{15}ClN_4]$ and $[FeC_{12}H_{15}ClN_4]$ complexes, it

appeared at 1656.52 and 1647.26cm^{-1} respectively. These shift suggest the involvement of C=N in complexation to Ni and Fe respectively. The vibration stretching frequency of C=N in [$CoC_{12}H_{15}ClN_4$] appeared at 1637.66 cm^{-1}. This indicate the non-involvement of C=N in coordination to cobalt.

In the electronic spectrum of pyrimethamine, the absorption maxima were observed at 198.41, 248.08, 278.65 and 295.47 nm. These absorption bands were assigned n-π* and π-π* respectively. These transitions are due to intraligand charge transfer (ILCT). The absorption maxima in the electronic spectrum of [$NiC_{12}H_{15}ClN_4$] were observed at 191.53, 255.94 and 263.28 nm were assigned n-π* and π-π* (ILCT) and the band at 295.43 nm were due to ligand to metal charge transfer (LMCT). The electronic absorption bands of [$FeC_{12}H_{15}ClN_4$] complex were observed at 193.06, 208.34, 240.44, 255.73, 272.54 and 294.70 nm. These bands were assigned n-π*, π-π* (ILCT) and ligand to metal charge transfer (LMCT). The absorption maxima of [$CoC_{12}H_{15}ClN_4$] observed at 194.59 and 240.44 nm suggested n-π*, π-π* (ILCT). The bands 254.96, 271.78 and 287.06 nm in the electronic spectrum of [$CoC_{12}H_{15}ClN_4$] suggested ligand to metal charge transfer (LMCT).

The zone of inhibition of pyrimethamine against *Nesseria gonorrhea and Proteus mirabilisat at* 250.00, 125.00 and 65.25 mg/l showed that it was significantly lower ($P<0.05$) than the complexes. The zone of inhibition of [$FeC_{12}H_{15}ClN_4$] against *Nesseria gonorrhea and Proteus mirabilisat at* 250.00, 125.00 and 65.25 mg/l showed that it was significantly higher ($P<0.05$) than [$NiC_{12}H_{15}ClN_4$] and [$CoC_{12}H_{15}ClN_4$]. This suggests that the complexes were more potent than the parent ligand against the bacteria strains used.

Based on the electronic and infrared spectrum analysis, the following structures (Figure 10) were proposed for the complexes.

CONCLUSION

Pyrimethamine complexes of Ni(II), Fe(II) and Co(II) have been synthesized. The chelating agent behaved as a tetradentate ligand towards nickel and iron and behaved as bidentate ligand towards cobalt ion. The complexes were more potent than the parent ligand against *Nesseria gonorrhea and Proteus mirabilisat.*

ACKNOWLEDGMENTS

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